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Comparison of three algaecides for controlling the density of *Prymnesium parvum*

John H. Rodgers, Jr. *, Brenda M. Johnson, West M. Bishop

Department of Forestry and Natural Resources, Clemson University

261 Lehotsky Hall, Clemson, SC 29634

*Corresponding Author. Tel.: 864 656 0492; Fax: 864 656 1034

e-mail address: jrodger@clemson.edu

Authors: Respectively, Professor, Graduate Research Assistant, Graduate Research Assistant.

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ABSTRACT

Prymnesium parvum has become more prevalent in water resources of the southern United States. Since the potential impacts of *P. parvum* are relatively well known, especially its capability to severely affect fish, managers have sought efficacious, environmentally sound and socially acceptable strategies for mitigating this noxious species. Laboratory testing was used to identify an effective algaecide for control of *P. parvum* from Texas, Arizona, Florida, North Carolina and South Carolina. Cutrine®-Plus at 0.2 mg Cu/L significantly decreased the density of *P. parvum* in samples from all of these locations. Both copper sulfate and Phycomycin® were less effective for controlling the population growth of *P. parvum*. The predicted response from the laboratory study was confirmed in the field at the Arizona site. Strategic use of Cutrine®-Plus in larger water resources could provide toxin-free refugia to allow some fish to survive and repopulate the water resource when the golden alga infestation abates.

KEY TERMS: algae, *Prymnesium parvum*, fish, toxicology, environmental impacts, treatment, copper, peroxide, toxin, mitigation

INTRODUCTION

Prymnesium parvum N. Carter is commonly referred to as the "golden alga." This haptophyte protist (Green and Leadbetter, 1994) is a relatively small (~10 microns), generally halophilic organism that intermittently produces an ichthyotoxin. This organism is widely distributed and has been implicated in numerous and extensive fish kills in brackish waters and inland waters with relatively high mineral content on five continents (Guo et al., 1996; Holdway

et al., 1978; James and de la Cruz, 1989; Kaartvedt et al., 1991; Lewitus et al., 2003; Lindholm et al., 1999; Otterstrom and Steemann-Nielsen, 1940) posing a difficult challenge for managers of some critical water resources.

Prymnesium parvum poses unusual problems as a planktonic organism (Graneli, 2006; Sunda et al., 2006; Uronen et al., 2007). Most noxious algae or planktonic organisms cause problems such as formation of suspended solids, increased oxygen demand and pH shifts in lentic waters due to dense growths as well as production of taste and odor compounds. *Prymnesium parvum* produces a suite of toxins called prymnesins that have ichthyotoxic, cytotoxic, and hemolytic effects (Shilo 1971; Shilo 1981). The toxins have been relatively difficult to isolate and characterize. The lack of a strong correlation between *P. parvum* cell density and toxicity (Shilo, 1981) is likely due to enhancement of toxicity or potency by several environmental factors such as temperature < 30°C (Baker et al., 2007; Shilo and Aschner, 1953), pH > 7.0 and phosphorus (Shilo, 1971) or nitrogen limitation (Graneli and Johansson, 2003). Although dense growths of *P. parvum* may color the water yellow to copper-brown or rust, massive fish kills may not be accompanied by visibly notable "blooms" of golden algae. The *P. parvum* ichthyotoxin affects gill-breathing aquatic animals including fish, brachiated tadpoles and mollusks (Shilo, 1967) and causes loss of selective permeability of gill epithelial cells and subsequent mortality (Ulitzer and Shilo, 1966; Shilo, 1967).

Periodic or intermittent production of ichthyotoxin may complicate a risk-based decision regarding intervention when faced with an encroachment or "bloom" of *P. parvum* in a critical water resource. But the record of massive fish kills associated with this haptopyte protist compels the decision to intervene if some criteria are met (e.g. early detection, ability for rapid response, potential maintenance of *P. parvum*-free refugia for fish and other potentially affected

organisms, etc.). When problematic algae interfere with critical water resource usages and immediate response is required, algaecides can often provide relief and rapidly restore the usages.

In order to support water resource management and discern potential tactics that can be used to control outbreaks of *P. parvum*, additional information is needed regarding exposures of algaecides that may control the population growth of these haptophytes. The objectives of this research were: 1) to verify ichthyotoxin production by samples of *P. parvum*; 2) to measure responses of samples of *P. parvum* from five sources to algaecide exposures in laboratory tests; 3) to assess the potential for use of an algaecide in field situations; and 4) to confirm algaecide effectiveness in a field application.

MATERIALS AND METHODS

Samples of *P. parvum*. Water samples for this study containing *P. parvum* were collected from: 1) Lake Whitney near Clifton, Texas (31° 57' N/97° 24' W), 2) Water Ranch Lake near Gilbert, Arizona (33° 22' N/111° 45' W), 3) Stormwater Lake near Sarasota, Florida (27° 19' N/82° 32' W), 4) City Lake near High Point, North Carolina (35° 51' N/80° 12' W), and 5) Stormwater Pond near Socastee, South Carolina (33° 41' N/79° 00' W). These sites are designated TX, AZ, FL, NC and SC, respectively. From each site, composite samples of at least 15 liters of water were collected about 20 cm below the surface (since *P. parvum* is sensitive to UV radiation; Smith, 2005). Both cell counts (microscopy) and an ichthyotoxin bioassay were used to confirm the presence and activity of *P. parvum*. The water samples were stored on ice

and shipped expeditiously to the laboratory at Clemson University where analyses commenced upon arrival.

Ichthyotoxin production by samples of *P. parvum*. A bioassay involving fish was used to estimate *P. parvum* activity in terms of ichthyotoxin production (Sager et al., 2007). This bioassay identifies waters with sufficient toxin (or are developing sufficient toxin concentrations) to pose potential risks to fish in the water resource and is useful for evaluation of the intensity of ichthyotoxin production to aid a decision regarding algaecide treatment. The assay involves exposing fish (larval *Pimephales promelas*, fathead minnow) to the water in question as well as dilutions of that water. The fish used were <24 hour – old larvae and were obtained from a culture in the Clemson University Aquatic Laboratory (Johnson et al., 2008). In the assay, the *P. parvum* ichthyotoxin potency can be augmented by the cation DADPA (3,3-diaminodipropylamine) which serves as a cofactor or promoter in laboratory tests by increasing the sensitivity of fish to toxicant already present in the water (Ulitzer and Shilo, 1966; Ulitzer and Shilo, 1964). For assessment of these samples, the promoter was not used since the <24 hour – old larval *P. promelas* were relatively sensitive to the *P. parvum* ichthyotoxin. Four replicates, containing ten fish per replicate, containing 200 mL of water in 250 mL beakers were used for each dilution (100, 50, 25, 12.5, and 6.25%) of sample water along with a control consisting of moderately hard laboratory water (Johnson et al., 2008). Water characteristics were measured prior to testing (Table 1). As recommended in Sager et al. (2007), all assays were conducted at 28°C.

Responses of samples of *P. parvum* to algaecide exposures in laboratory tests.

Preparation of stock algaecide solutions for laboratory experiments. Three algaecides (copper sulfate pentahydrate, Cutrine®-Plus, and Phycomycin®) were evaluated for efficacy in controlling samples of *P. parvum* (Table 2). Copper sulfate pentahydrate (~25.5 % copper; Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178 USA) is a non-chelated form of copper and is representative of the common copper salt used as an algaecide. Cutrine®-Plus is a chelated-copper containing compound (Cu_2CO_3), with an elemental copper concentration of 9% in the form of mixed copper - ethanolamine complexes (Applied Biochemists, 2007a). Stock algaecide solutions for treatments with copper sulfate pentahydrate and Cutrine®-Plus (100 mg Cu / L) were prepared using NANOpure™ water within four hours prior to experiment initiation. Phycomycin® is sodium carbonate peroxyhydrate (Applied Biochemists, 2007b) and was applied directly in granular form to initiate these treatments in the laboratory experiments.

Algal toxicity experiments. Algal toxicity experiments evaluating copper sulfate pentahydrate and Cutrine®-Plus were initiated using four replicates of treatment concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg Cu / L as algaecide, and an untreated control (Fitzgerald and Jackson, 1979; Murray-Gulde et al., 2002; USEPA, 2002). Experiments with Phycomycin® used four replicates of treatment concentrations of 0.5, 1.0, 3.0, 5.0, and 10.0 mg H_2O_2 / L as algaecide, and an untreated control. Experimental chambers consisted of static, non-renewal 200 mL volumes of treated or untreated site waters containing *P. parvum* in Erlenmeyer flasks and were swirled once daily by hand. Exposure duration for each experiment was 96 hours. A 16:8 hour light-dark cycle was used, with "cool white" fluorescent lighting at an intensity of 86 ± 8.6 µE / m^2 / s. Exposure chambers were maintained at a temperature of 22 ± 2 ℃ (modified from APHA, 1998). Total copper concentrations were measured using a Perkin-Elmer 5100 PC flame

and graphite furnace atomic absorption (AA) spectrometer (method 3010-B; APHA 1998). Water characteristics (pH, dissolved oxygen, conductivity, total alkalinity, total hardness, total nitrogen, total phosphorus, and temperature) were measured according to APHA (1998) methods.

Algal Response Parameters. Response parameters measured for the *P. parvum* -algaecide toxicity experiments included chlorophyll *a* concentrations and cell densities. Chlorophyll *a* was extracted according to United States Environmental Protection Agency (USEPA) method 445.0 (Arar and Collins, 1997) and measured using a SpectraMax® 190 Gemini 96 well plate spectrofluorometer (Molecular Devices Corporation, Sunnyvale, CA 94089). Fluorometeric measurements of test samples were calibrated using chlorophyll *a* standards prepared from a stock solution of 4000 µg chl *a* /L (Sigma C-5753; range = 10-1280 µg chl *a* /L), kept protected from light in covered bottles and stored at -20°C. *Prymnesium parvum* densities were measured using light microscopy with a Leitz Dialux 20 microscope and an Improved Neubauer hemacytometer at 400X magnification (Southard, 2005).

Assess the Potential for Use of an Algaecide in Field Situations. In addition to efficacy for the target species, an important consideration for field use of algaecides is the margin of safety (MOS) for non-target species (Murray-Gulde et al., 2002). Effective algaecide concentrations for control of *P. parvum* were compared to toxicity data for sensitive, sentinel non-target species of fish and invertebrates to calculate margins of safety. For this study, the margin of safety was calculated as follows:

$$\text{MOS} = \frac{\text{Effective concentration for adverse effects on non-target organism}}{\text{Effective concentration for control of } P.\ parvum} \qquad \text{Equation 1}$$

Thus a MOS ≤ 1 indicates potential for risks for non-target species, while a MOS > 1 indicates less potential for adverse effects to non-target species.

Confirm Algaecide Effectiveness in a Field Application. Application of algaecide for control of *P. parvum* in Water Ranch Lake (Gilbert, AZ) provided an opportunity to confirm the prediction from the laboratory testing. Water Ranch Lake is about 2.0 hectares (5 acres) with an average depth of 3.4 meters (~11 feet) and is located at an elevation of 340 meters (~1,100 feet). Sport fishing is important for this water resource (Arizona Game and Fish Department, 2007). Cutrine®-Plus was applied at a target concentration of 0.2 to 0.25 mg Cu/L. Responses of *P. parvum* were measured along with responses of non-target fish.

Statistical Analysis. A one-way analysis of variance (ANOVA) was used to determine differences in chlorophyll *a* concentrations and cell densities between treatments and untreated controls. Differences were discerned further using Dunnett's multiple range test. If data did not meet the assumptions for parametric testing, then a non-parametric ANOVA on ranked data was used followed by Dunn's multiple range test. All data were analyzed using SigmaStat version 3.1 for Windows (alpha = 0.05) (Systat Software, Inc., Point Richmond, CA 94804-2028).

RESULTS AND DISCUSSION

Ichthyotoxin production by samples of *P. parvum*. Ichthyotoxin was detected in all samples of *P. parvum* except the sample from City Lake near High Point, NC (Table 3). Although variance in *P. parvum* density was not sufficient to discern a correlation between cell

density and ichthyotoxin production, the sample from NC with no measurable ichthyotoxin had the lowest cell density (3.8 x 10^3 cells/mL) of the samples tested. The ichthyotoxin content of samples with *P. parvum* densities ranging from 1.4 x 10^4 cells/mL to 4.7 x 10^4 cells/mL was relatively potent with survival of *P. promelas* larvae in three of four samples only after dilution of the water samples to 6.25%. Approximately 50% survival of fathead minnow larvae was observed in the sample from FL after dilution to 12.5%. The acute responses of fathead minnow larvae to exposures in waters containing *P. parvum* in the absence of cofactor were indicative of the potency of the ichthyotoxin at these sites.

Responses of samples of *P. parvum* to algaecide exposures in laboratory tests.

Measured concentrations of copper in treatments were within 12% of targeted concentrations, so all results are reported as targeted concentrations. Water characteristics for the samples were similar, except for the NC sample with notably lower ionic strength (Table 1). The water characteristics of these reservoirs and stormwater lakes supporting *P. parvum* populations were indicative of relatively nutrient rich systems that were moderately hard to hard waters except for the reservoir in NC. This reservoir had been experiencing a period of drought during the time of this sampling. All of the samples of *P. parvum* were relatively sensitive to exposures of Cutrine®-Plus with > 90% reduction in cell density at concentrations of 0.2 mg Cu/L (Figure 1). The responses of these samples of *P. parvum* to copper sulfate and Phycomycin® were significantly less in terms of cell density than for Cutrine®-Plus. Chlorophyll *a* concentrations in treatments declined (Figure 2) paralleling results observed for *P. parvum* cell density. Cutrine®-Plus was significantly more effective than copper sulfate or Phycomycin® in terms of decreasing chlorophyll *a* concentrations. Both copper sulfate and Phycomycin® were less effective for

controlling the population growth of *P. parvum*. Samples treated with Cutrine®-Plus at concentrations ≥ 0.2 mg Cu/L were examined 14 days after treatment and no regrowth of *P. parvum* cells was observed. Since the other algaecides were not as effective for controlling *P. parvum*, regrowth was not assessed.

Assess the Potential for Use of an Algaecide in Field Situations. Often an important decision criterion for use of an algaecide to respond to an outbreak of *P. parvum* is the MOS for non-target species such as fish. For this study, MOS was defined as the ratio of the concentration of algaecide that adversely affects a sensitive, sentinel non-target fish and the concentration required to control the population growth of *P. parvum*. A MOS ≤1 indicates risk to non-target species, while a MOS >1 indicates less potential for adverse effects to non-target species. For this situation, we estimated a MOS of 3.5 based upon the ratio of the lowest observed effect concentration (LOEC) for a 96-hour exposure of < 24-hour old *Pimephales promelas* to Cutrine®-Plus (0.75 mg/L) and the concentration of Cutrine®-Plus required to control the growth of *P. parvum* cells (0.2 mg/L). Aquatic invertebrates are equally or more sensitive to exposures of Cutrine®-Plus, however a MOS > 1 exists for many of these species (Table 4). Water resource managers considering applications of copper formulations for control of *P. parvum* must also consider the relative risks and mitigate these risks where possible.

Currently, algaecides registered by the US Environmental Protection Agency for use in water resources include: acrolein, copper formulations, diquat dibromide, endothall formulations, and peroxide formulations. The endothall formulations and acrolein are not compatible with maintenance of fish or other aquatic life (i.e. no MOS) and are used as biocides in situations such as irrigation canals. Diquat dibromide is not effective in aquatic systems that have suspended

particulates that can bind the algaecide and render it not bioavailable. The copper formulations registered as algaecides differ in degree of chelation as well as inclusion of adjuvants. Concomitantly, their algaecidal effectiveness varies widely (Murray-Gulde et al., 2002). As indicated above, some copper formulations have a MOS for non-target species such as fish (*P. promelas* MOS=4.3; Table 4) when site specific treatments are judiciously applied. Similarly, peroxide formulations have a MOS but were not effective for controlling the population growth of *P. parvum.*

The response of *P. parvum* to 0.2 to 0.25 mg Cu/L Cutrine®-Plus was rapid as was mitigation of the toxin production and effects. Other control tactics that have been used in ponds include treatment of *P. parvum* with ammonium sulfate (Barkoh et al., 2003). Ammonium sulfate concentrations required to control *P. parvum* (~0.17 mg /L of un-ionized ammonia) may produce unionized ammonia concentrations that adversely affect some fish (Barkoh et al., 2004). In this study, copper sulfate was not as effective for *P. parvum* as the chelated copper formulation. Neither barley straw nor Liquid Live Micro-Organisms™ were effective for control of *P. parvum* in pond situations in Texas (Barkoh et al., 2008). In laboratory tests (Grover et al., 2007), barley straw extract was also ineffective for *P. parvum* control, but relatively high treatments of ammonium (0.72 mg NH_4-N /L) were successful. Caution was offered regarding potential adverse effects of relatively high concentrations of ammonia on non-target species. Repeated treatments of ammonium chloride and phosphoric acid were somewhat successful for controlling *P. parvum* growth in limnocorrals in aquaculture ponds (Kurten et al., 2007), however the authors again indicated the potential for adverse effects on non-target species. Suspended solids (mud), organic fertilizer (manure) and decreased salinity have also been used to control *P. parvum* in Chinese aquaculture of carp species (Guo et al., 1996) with the best

results from decreased salinity and ammonium sulfate. If application of an algaecide is indicated to mitigate risks from *P. parvum*, all regulatory approvals and permits must be obtained.

Confirm Algaecide Effectiveness in a Field Application. Use of site water with associated algae in the laboratory algal toxicity tests minimizes the potential for ambiguity in applying laboratory results directly to a field situation (Fitzgerald and Jackson, 1979). The mean water characteristics for Water Ranch Lake were pH 7.75, hardness 411 mg/L as $CaCO_3$, alkalinity 104 mg/L as $CaCO_3$, and conductivity 2700 $\mu S/cm^2$. The pre-treatment cell density of *P. parvum* was 4.6 x 10^4 cells/mL with a chlorophyll *a* concentration of 24 µg/L. Prior to treatment, ichthyotoxin production was likely present as indicated by the presence of dead fish and *P. parvum*. Within 24 – 48 hours after treatment, the cell density of *P. parvum* declined and was not detectable (<5 x 10^2 cells/mL), while the chlorophyll *a* concentration decreased to <10 µg/L. *Prymnesium parvum* was not detected in Water Ranch Lake for two months and there was no recurrence of fish mortality.

SUMMARY

Toxin production by *P. parvum* is intermittent possibly due to genetic, density or environmental factors (Graneli and Johansson, 2003). "Triggers" for *P. parvum* ichthyotoxin production are not known, but they are perhaps connected to drought and other factors that alter ionic strength and composition of waters since *P. parvum* is now found in inland waters with lower ionic strength and ion composition differing from seawater. Not all water resources with detectable densities of *P. parvum* have sufficient ichthyotoxin to cause fish kills. Sager et al.

(2007) noted that the golden alga can produce enough toxin to cause a fish kill when cell densities are as low as 1.0 x 10^4 cells/mL, but fish losses in Texas typically have not occurred until algal density achieved 2.0 x 10^4 cells/mL or more. Based on laboratory and field observations, *P. parvum* ichthyotoxin is apparently not persistent since fish mortalities do not continue in the absence of the golden alga (Barkoh and Fries, 2005). These characteristics provide an opportunity to develop cell-free and toxin-free refugia for mobile species such as fish. Fish that have been sublethally exposed to *P. parvum* ichthyotoxin recover quickly when removed to uncontaminated water during the early stages of intoxication (Shilo, 1967). Based upon field observations, fish also have the ability to detect and avoid the toxin if a toxin-free refuge is available (Sarig, 1971). Lakes and ponds smaller than a few hundred hectares can be treated with algaecides successfully, but treatment of the entire water resource may not be economically feasible for larger or more complex lakes or reservoirs. Mention of a control tactic for toxin producing algae in this paper does not constitute endorsement of an algaecide or any other tactic for a specific situation. Local extension agents and authorities can provide information regarding site specific permit requirements and restrictions. Strategic use of algaecide in larger water resources could provide toxin-free refugia to allow some fish to survive and repopulate the water resource when the golden alga infestation abates.

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Uronen, P., P. Kuupo, C. Legrand, and T. Tamminen, 2007. Allelopathic effects of toxic haptophyte *Prymnesium parvum* lead to release of dissolved organic carbon and increase in bacterial blooms. *Microbial Ecology* 54:183-193.

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414 Organisms (Fourth Edition). USEPA, EPA-821-R-02-013, Office of Water, Washington,
415 D.C.

Table 1. Characteristics of water samples containing *Prymnesium parvum* subjected to algaecide testing. Mean and (range) of four samples.

Sample Source	pH SU	Hardness mg/L as $CaCO_3$	Alkalinity mg/L as $CaCO_3$	Conductivity µS/cm	Dissolved Oxygen mg/L	Total Nitrogen mg/L	Total Phosphorus µg/L
TX	8.27	312	130	1604	8.2	0.5	17
	(8.10-8.58)	(260-328)	(106-148)	(1580-1710)	(7.8-9.0)	(0.2-1.4)	(1-28)
SC	7.65	140	110	3600	8.7	0.4	8
	(7.28-7.88)	(106-160)	(90-144)	(2400-3860)	(8.2-9.3)	(0.2-0.8)	(1-16)
AZ	7.75	411	104	2700	7.8	0.2	13
	(7.21-8.12)	(346-438)	(92-124)	(2460-2840)	(7.4-8.2)	(0.1-0.4)	(1-18)
FL	8.10	186	240	1860	8.6	0.4	9
	(7.10-8.28)	(162-218)	(180-260)	(1680-2210)	(8.2-9.2)	(0.2-0.8)	(2-12)
NC	7.75	40	50	125	8.8	0.2	3
	(7.16-7.90)	(30-50)	(42-56)	(85-138)	(8.0-9.5)	(0.1-0.5)	(1-8)

Table 2. Physical properties of Cutrine-Plus®, copper sulfate pentahydrate, and Phycomycin® (Applied Biochemists, 2007 a,b; Hohman & Martin, 1995).

Properties	Cutrine-Plus®	Copper Sulfate	Phycomycin®
% active ingredient	9.0 (elemental Cu)	25.4 (elemental Cu)	85 (as sodium carbonate peroxyhydrate)
Application rate	0.2-1.0 mg Cu/L	0.05-0.5 mg Cu/L	0.2-10.2 mg/L as H_2O_2
Formulation	copper-ethanolamine complex	$CuSO_4$-$5H_2O$	sodium carbonate peroxyhydrate
Chemical Class	chelated elemental copper (Cu_2CO_3)	copper salt	oxidating agent
Mode of action	cell toxicant	cell toxicant	cell toxicant
Water solubility (mg/L)	Complete	316,000	complete
pH	10.0-11.0	NA	10.4-10.6 at a concentration of 1% solution

Table 3. Survival of *Pimephales promelas* in *Prymnesium parvum* ichthyotoxin assays.

Sample Source	*P. parvum* Density (cells/mL)	% Survival *Pimephales promelas* in Sample Dilution (% Sample)					
		100%	50%	25%	12.50%	6.25%	0%
TX	1.4×10^4	0	0	0	0	42	100
SC	1.4×10^4	0	0	0	0	40	100
AZ	4.7×10^4	0	0	0	0	30	97
FL	2.8×10^4	0	0	35	47	65	100
NC	3.8×10^3	100	100	95	100	100	95

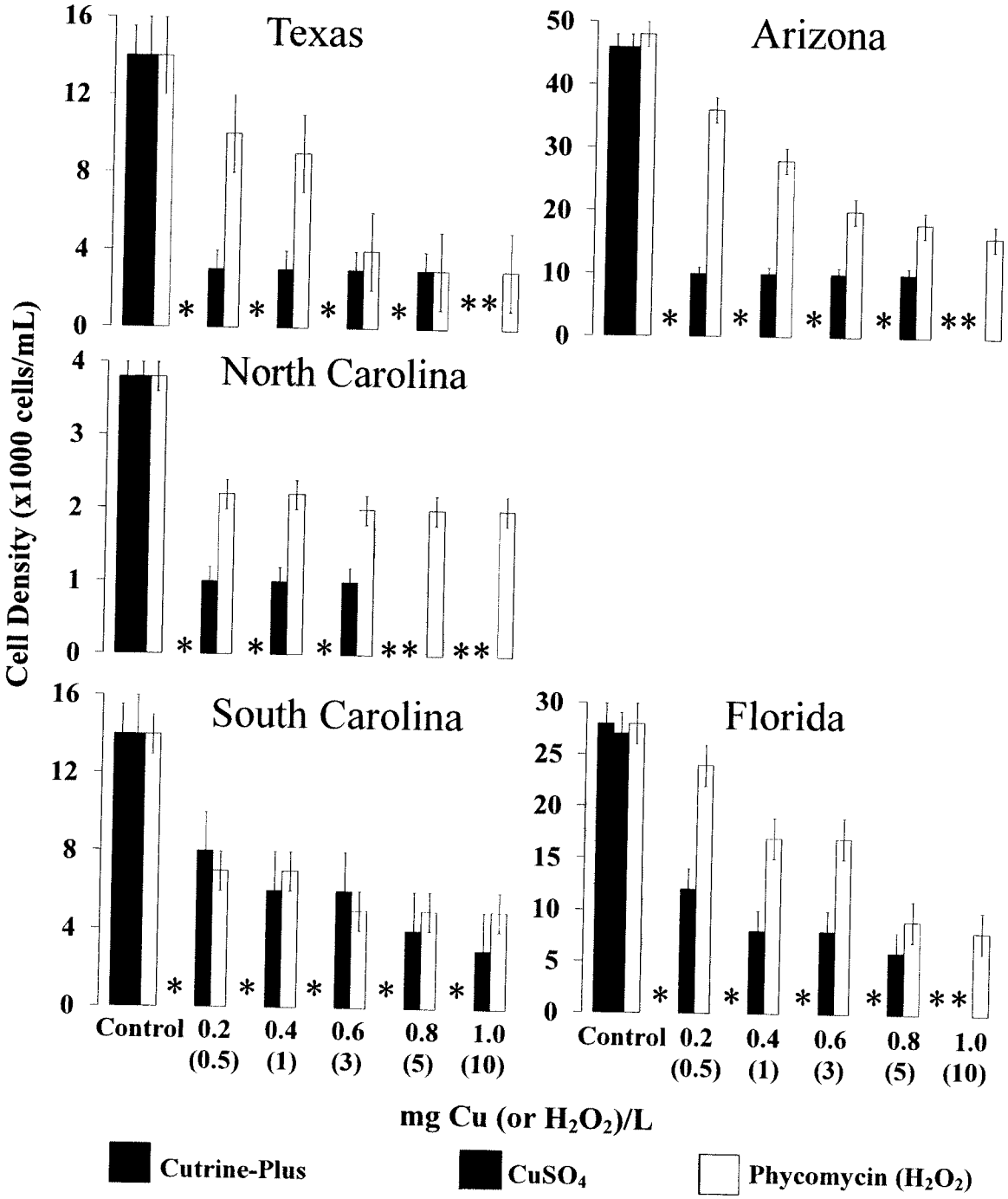
Table 4. Toxicity of Cutrine Plus® to sentinel non-target species and margins of safety associated with use of this algaecide. EC_{50} values (effective concentration for 50% of the population) are based on acid-soluble copper concentrations. Margin of safety was defined in Equation 1 [EC_{50} value divided by the concentration required to control the growth of *Prymnesium parvum* (200µg Cu/L)].

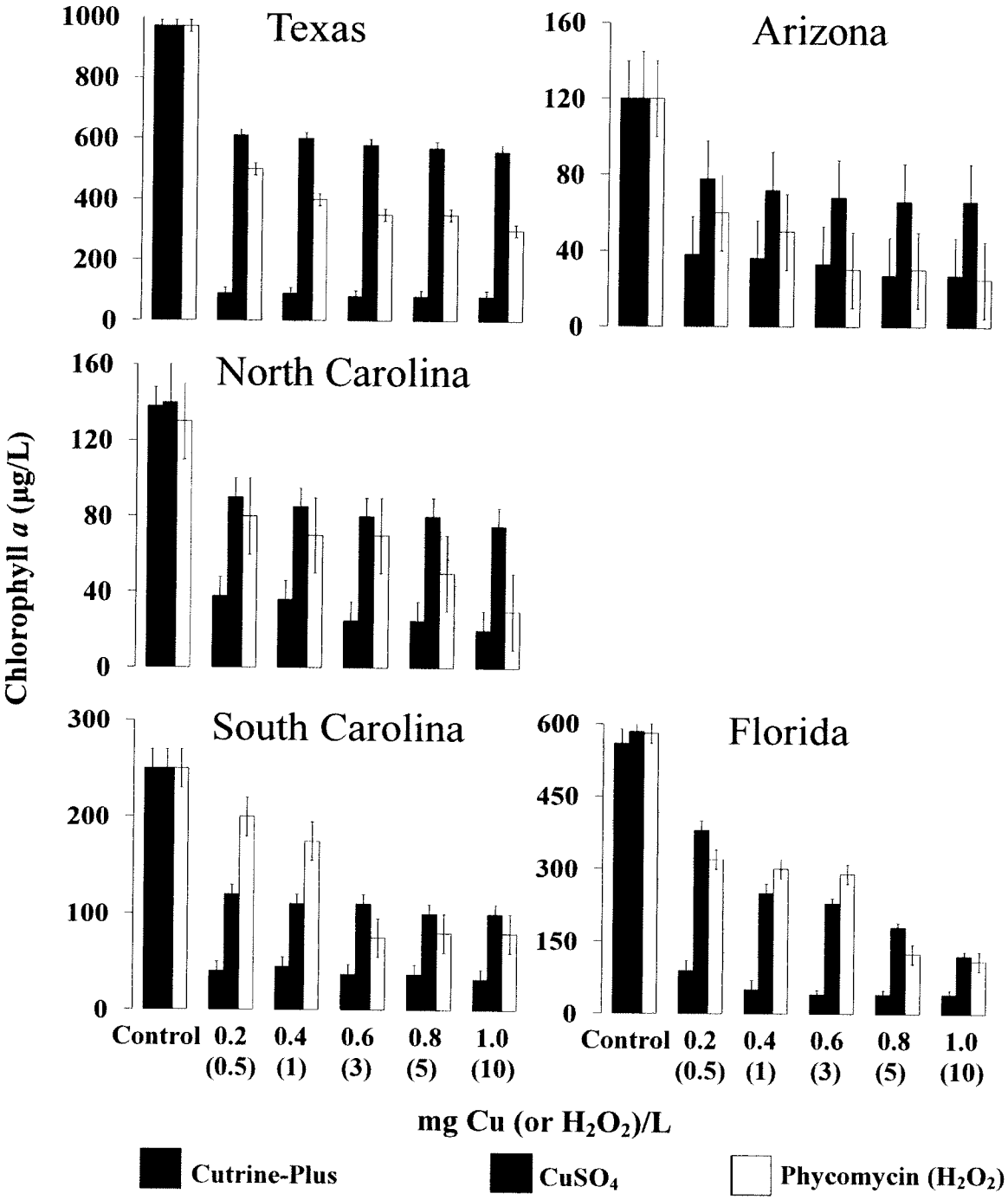
Organism	Test Duration (hours)	EC_{50} (ug Cu/L)	Margin of Safety	Citation
Ceriodaphnia dubia	96	124	0.6	Murray-Gulde et al. 2002[a]
Chironomus tentans[c]	48	460.9	2.3	Mastin and Rodgers 2000[b]
Hyalella azteca[d]	48	247.8	1.2	Mastin and Rodgers 2000[b]
Pimephales promelas	96	863	4.3	Murray-Gulde et al. 2002[a]
Lepomis macrochirus	96	13,300	66.5	Applied Biochemists 2007a[c]
Lepomis macrochirus	96	83,000	415	Applied Biochemists 2007a[d]

[a] Water chemistry (Murray-Gulde et al. 2002) alkalinity (18-424 mg $CaCO_3$/L), hardness (8-212 mg $CaCO_3$/L), conductivity (83-9,750µS/cm), pH (6-8.1)
[b] Water chemistry (Mastin and Rodgers 2002) alkalinity (55-96 mg $CaCO_3$/L), hardness (48-96 mg $CaCO_3$/L), conductivity (270-450 µS/cm), pH (6.4-8.0)
[c] *Chironomus tentans* is a benthic infaunal organism that constructs a case from organic material from which it feeds during its larval stage.
[d] *Hayella azteca* is an epibenthic detritivore that feeds on organic material on the sediment surface.
[c] Total Harness 48 ppm
[d] Total Hardness 200 ppm

Figure 1. Responses (cell densities) of *Prymnesium parvum* in samples from Texas, Arizona, Florida, North Carolina, and South Carolina to 96-hour exposures of Cutrine®-Plus, copper sulfate pentahydrate, and Phycomycin®. * indicates Non-Detect value (<1000 cells/mL). Error bars represent one standard deviation.

Figure 2. Responses (chlorophyll *a* concentrations) of *Prymnesium parvum* samples from Texas, Arizona, Florida, North Carolina, and South Carolina to 96-hour exposures of Cutrine®-Plus, copper sulfate pentahydrate, and Phycomycin®. Error bars represent one standard deviation.


Texas
Arizona
North Carolina
South Carolina
Florida
Cell Density (x1000 cells/mL)
Control
0.2 (0.5)
0.4 (1)
0.6 (3)
0.8 (5)
1.0 (10)
mg Cu (or H_2O_2)/L
Cutrine-Plus
$CuSO_4$
Phycomycin (H_2O_2)


Texas
Arizona
North Carolina
South Carolina
Florida
Chlorophyll a (µg/L)
1000
800
600
400
200
0
160
120
80
40
0
300
100
600
450
150
Control
0.2 (0.5)
0.4 (1)
0.6 (3)
0.8 (5)
1.0 (10)
mg Cu (or H_2O_2)/L
Cutrine-Plus
$CuSO_4$
Phycomycin (H_2O_2)